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PROCEEDINGS

High-Resolution Microbial Pitting Corrosion Studies Utilizing a Two Dimension Scanning Vibrating Electrode Microscope (SVEM) System.

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Abstract

Traditionally, electrochemical techniques used in corrosion studies have been limited by their lack of spatial and temporal resolution. Microbial corrosion is often evidenced as pitting in which small anodes and correspondingly large cathodes are formed. Techniques such as electrochemical impedance spectroscopy, which are unable to differentiate the anode form the cathode, therefore underestimate the rate of corrosion due to the fact that it averages both anodic and cathodic processes. Vibrating micro-electrodes allow mapping of the current densities over a corroding metal surface where the resolution is determined by the height of the electrode above the surface (resolution =twice the height) and the number of measurements made. This theoretical value is approached by vibrating the electrode in the vertical plane as well as the horizontal plane helping to smooth out irregularities in the surface topography. By scanning such an electrode over the surface, a current density map can be generated showing the size and location of both the anodic and cathodic regions with time. Due to the rapid nature of the scans (<5 minutes), the temporal resolution is also enhanced.

A system giving a resolution of approximately 300 μm has been coupled with a microscope (resolution better than 1 μm) and a photon counting imaging system capable of detecting the light produced by a single luminescent bacterium. This system allows the congruent mapping of bacterial locations and activity (bioluminescence) with the resultant current

densities. It has been shown that anodic regions are formed below colonies of bacteria. Manipulation of these colonies of bacteria have shown that 10^6 cfu cm^{-2} are necessary to form an anodic region. Further investigations, including the incorporation of genetically engineered "reporter bacteria" containing the luxB gene will allow the determination of the mechanisms whereby microbial corrosion takes place. The proximity between components of a microbial consortium necessary for inducing MIC can be determined as well.

Introduction

Microbial corrosion of metals normally entails the establishment of an area of low potential (anodic region) relative to a corresponding area of high potential (cathodic region) on the surface. As current flows from the area of high potential to low potential metal ions are lost from the surface, when the anodic region in maintained and localized in one region a pit is formed. Due to the localized nature of pitting the area of attack is normally quite small, less than 5 mm in diameter.

A number of methods of microbial attack on metal surfaces have been postulated [1]. Of these the most studied has involved the action of the sulphate reducing bacteria (SRB) on mild steel. However, it needs to be noted that microbially influenced corrosion is not limited to SRB and mild steel. MIC has been reported for numerous metals and metal alloy as reviewed by Little et al., [2]. Even the relatively toxic copper compounds have been shown to be vulnerable to MIC in fresh water [3].

As pitting is an electrochemical process the magnitude of the current flowing can be measured by electrochemical techniques, including linear polarization and electrochemical impedance spectroscopy. However, due to the relative small size of the active regions in relation to the total area of the test coupon the magnitude of the rate of corrosion is often under estimated. These methods also are unable to provide any information on the spatial correlation between any microorganisms possibly involved in the process and the site of attack.

A system has been described in the literature which allows for the mapping of local current densities to be measured in a conductive solution above a metal surface [4],[5]. The system is based on the ability of a vibrating electrode to capacitively detect a potential drop between its two extremities of oscillation. This potential drop is determined by the local current density at that point in the solution. In order to achieve a high

resolution of the current densities a platinum black coated stainless steel or platinum microelectrode with a tip diameter of 20-30 μm is utilized. With such a microelectrode positioned close to a metal surface it is possible to achieve a resolution in the region of 100 - 300 μm. By running the system under the control of fast modern microcomputers it is possible to quickly scan relatively large areas, for example an area of 25 mm^2 can be scanned with a resolution of 15 x 15 data points in approximately five minutes. This allows for good temporal resolution to be achieved as well as the spatial resolution.

Franklin et al. [5] utilizing such a system, with the probe being vibrated in one plane parallel to a mild steel surface, showed that the presence of a culture of Pseudomonas sp. could prevent the repassivation of locally formed anodic regions seen in sterile controls. Although this system was able to show the localized nature and magnitude of the anodic regions associated with bacterial activity it was unable to provide information on the spatial relationship between the bacteria and the anodic region.

Davenport et al [6] showed that the resolution of the vibrating electrode could be enhanced nearer it's theoretical maximum by vibrating the probe in the vertical plane at the same time as the vertical plane. Angell et al. [7] took the system a stage further by incorporating the microelectrode with a microscope fitted with a CCD camera system capable of detecting the light emitted by a single bacterium. They were able to demonstrate that the anodic region formed below a "pseudo" colony of bacteria placed on a mild steel coupon. This system was, however, static with no nutrient feed and the anodic region was maintained for less than twenty four hours.

The bacteria used by Angell et al. [7] had been isolated from a corrosion tubercule formed on a stainless steel pipe carrying fresh water in a power generation plant. These bacteria had been genetically manipulated to carry in a plasmid the luxB gene inserted into certain pathways of interest. It has been shown using such methods that the production of light can be correlated to the initiation of the pathway of interest [8].

This paper will show the way in which this powerful tool, the scanning vibrating electrode microscope (SVEM), has been used in order to further understand the mechanisms of pitting in mild steels by non sulphate reducing bacteria.

Materials and Methods

Bacteria:

A genetically engineered bacteria, containing the plasmid PUTK50, was primarily used in this study. PUTK50 is a plasmid containing the luxB gene which encodes for bioluminescence [9]. The bacteria was Pseudomonas aeruginosa FRD1 isolated from the lungs of a cystic fibroses patient. This bacteria was chosen due to its ability to produce copious amounts of the exopolymer (EPS) alginate.

Medium:

The medium used was chosen for its ability to enhance polymer production in biofilm grown cells as determined by both light production and EPS assays. It should be noted that this requirement often resulted in a sub optimal medium from the corrosion point of view, with excess sodium chloride being present. For the FRD1 experiments the medium contained in (g L^{-1}): glutamic acid 0.015, glycerol 0.045, magnesium sulphate 0.009, sodium chloride 8.5, Kanomycin 0.05, Carbenicillin 0.05 and 1 ml of phosphate buffer containing (g L^{-1}): 20.8 sodium orthophosphate, 58.6 di-potassium phosphate.

SVEM:

A two-dimensional scanning vibrating electrode microscope (2D-SVEM) utilizing a 2 channel lock-in amplifier system was used. A four axis motion system allowed movement of the probe in the X, Y plane, and Z plane as well as focusing the Zeiss Axioplan microscope in the W plane. The microscope allowed visual images, both bright field and photon, to be collected via a Hamamatsu C2400-47 photon-detecting camera attached to the microscope. This system was found to work well with the long working distance objectives available allowing both low power images of a 5 x 5 mm area (2.5 x obj.) as well as high power images viewing individual bacterium (40 x obj.) All the images were processed by the Argus 10 image analyzer before being sent to the computer for use by the VP software (ScienceWares, Woods Hole, MA). The VP software controlled the location and movement of the probe relative to the visual image and logged the local current densities at each sampling site. This information could then either be plotted congruently with the visual image as a surface contour plot, or both could be exported to other software programs with more sophisticated plotting routines. Stainless steel probes electrolytically coated with platinum black were used through out this experiments. At the start of each experiment each electrode was calibrated using a glass pipette drawn to a

fine point filled with potassium chloride and coupled to a Ag/AgCl half cell. A known current was passed through this calibration electrode at a known distance from the probe, in both the x and y planes, allowing the computer to make calibrations of the probe for current densities. It should be noted that the probe actually measures the local current density in the solution at its operating height above the metal coupon. This is not the current density at the surface as there is a gradient away from the surface.

Electrochemical DC polarisation measurments were conducted by Softcorr corrosion measurment software associated with EG & G model 273 (option 92) potentiostat. For linear polarisation resistance, current densities were monitored within ±30 mV verses open circuit potential with a scan rate of 0.17 mV/sec.

Specimens:

Test specimens used were 16 mm diameter disks of AISI C1020 carbon steel. These disks were mounted into epoxy resin casts and wet-polished in sequence with 240, 400, and 600 grit SiC paper, ultrasonically cleaned with distilled water, degreased with acetone and sterilized with 70 % propan-1-ol for 30 minutes and allowed to air dry in a laminar flow hood. All other tubing and containers were sterilized by autoclaving at 121 °C and 15 PSI for 20 minutes.

Results and Discussion

Two sets of data will be presented that are representative of two variations used for inoculating the system. First a twenty-four hour broth culture was added as the bulk phase, allowing random colonisation over the whole surface. Second 25 ml of a twenty-four hour broth was spun down and resuspended in 250 µl of phosphate buffered saline (PBS) and a 1 µl drop of this concentrated bacterial solution allowed to sit on the coupon for thirty minutes. This method promoted localised attachment over a small area approximately 1 mm in diameter. PBS was used as it allowed slight evaporation to take place without increasing the salt level too much for the bacteria.

Figure 1 shows a series of scans over a forty-two hour period of the current density measure perpendicular to the surface of the coupon, when the bacteria had been applied to the bulk phase. Initially when the bacteria were added (1a) there was one large anodic spike, it is likely that this is a noise artifact as it was only one point. It is also unlikely that the bacteria

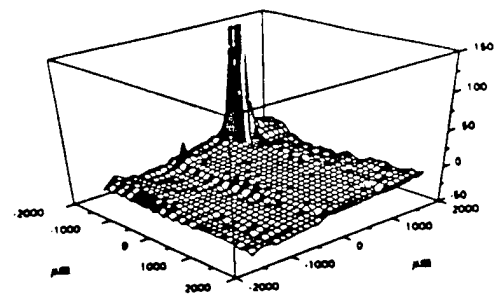

Figure 1a. (Time = 0 hr)

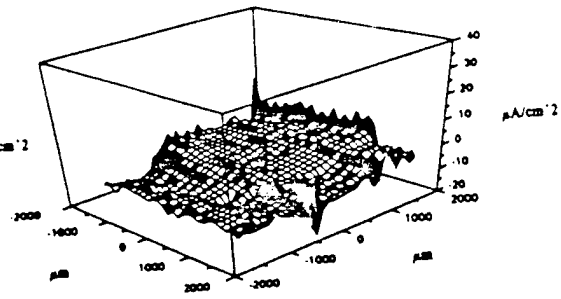

Figure 1b. (Time = 1hr)

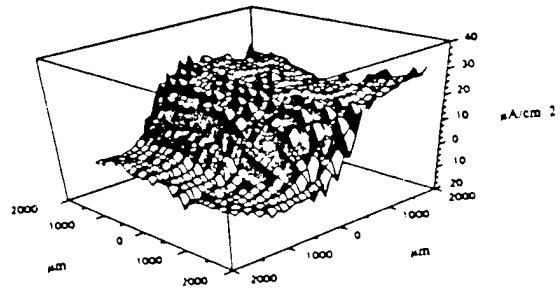

Figure 1c. (Time = 19 hr)

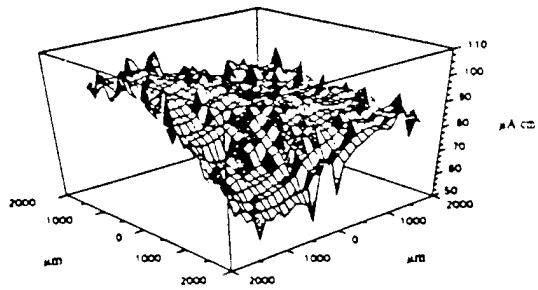

Figure 1d. (Time = 22 hr)

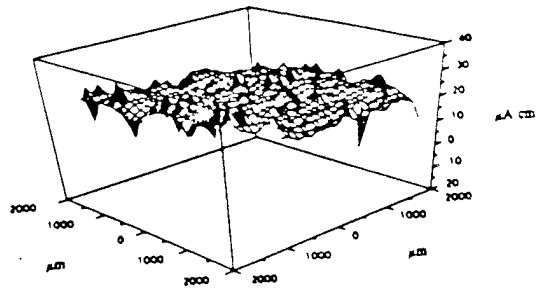

Figure 1e. (Time = 30 hr)

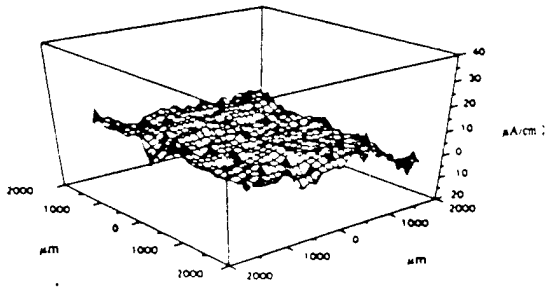

Figure 1f. (Time = 42 hr)

Figure 1. Current density (Z axis) scans resulting from the addition of a 24 hr broth culture of Pseudomonas aeruginosa FRD1 was added to the bulk phase over a mild steel coupon.

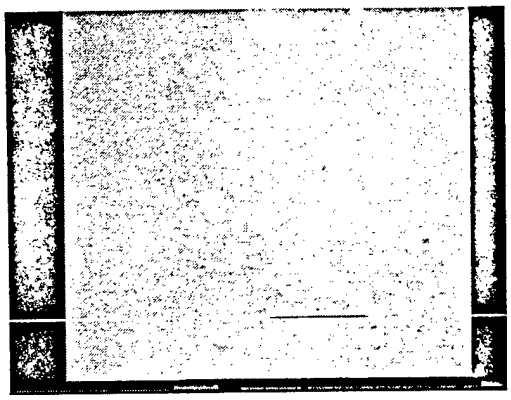

Figure 2a.

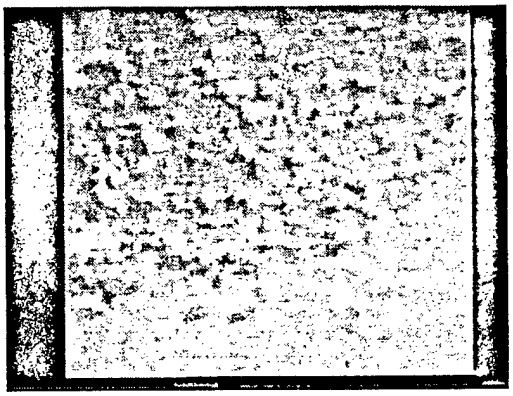

Figure 2b

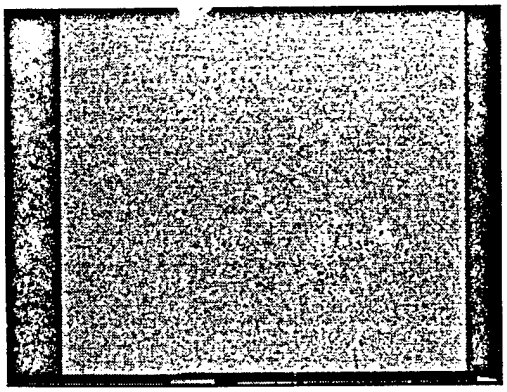

Figure 2c.

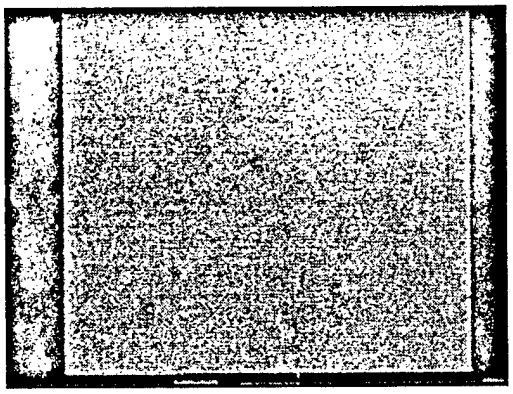

Figure 2d

Figure 2. Images of Pseudomonas aeruginosa FRD1 on mild steel coupon: a) visual image after 1 hour exposure, b) visual image after nineteen hours, c) photon image after 1 hour showing little light, b) photon image after nineteen hours showing more light.

would have had sufficient time to really start to attach to the surface and be exerting an effect. After one hour (1b) there appeared to be a slight increase in the anodic current over roughly two-thirds of the coupon. This anodic region had grew in magnitude until nineteen hours (1c). By twenty -two hours (1d) this anodic region had shifted slightly and covered most of the area being observed. However, after thirty hours (1e) the anodic region has disappeared with the plot showing general noise. This was similar to the resultant scan seen at forty-two hours (1f).

The corresponding images for the above scans are shown in Figure 2. These show both "brightfield" images where the image area is the same as the scans approximately 4.5 mm x 3.5 mm. These images were collected by the Hamamatsu C2400-47 camera with the gain turned down, the images appear hazy due to the image intensifier, that gives very high light sensitivity but poor spatial resolution. The other form of image is collected with the gain set on high allowing single photons to be collected. The image was acquired for five minutes. In order to remove the background noise counts were made with no bacteria present and the Argus 10 manipulated to drop out all the noise. This resulted in the loss of some dimmer bacterial light from the final image but at least allowed the brighter bacterial areas to be detected with out any background insuring the light seen on the screen was from bacteria.

Figure 2a is the visual image taken after one hour, showing that some material settling on the surface of the coupon. The larger light coloured amorphous material is thought to be alginate. Figure 2b shows another brightfield image this time taken after nineteen hours, This time the surface has been covered by a "fluffy" type material thought to be an iron oxide, mixed with alginate. This picture corresponds to the point when the anodic region was covering most of the coupon (1c). Looking at the photon images it is evident that after 1 hour there is very little light being produced by the bacteria (2c). By nineteen hours (2d) there was a lot of light with a number of bright areas indicating the presence of bacteria with the alginate pathway activated. This level of light was maintained until the end of the experiment at forty-two hours indicating that the bacteria were still alive and active, even though the anodic region had been passivated.

When a drop of concentrated bacteria was added to a localised area the results were as shown in Figure 3. After 1 hour a localised anodic region was present (3a) and was seen to correlate with the visual image collected at the same time (4a) where the bacteria and their associated polymers are seen as the light central area on the metal. After three hours

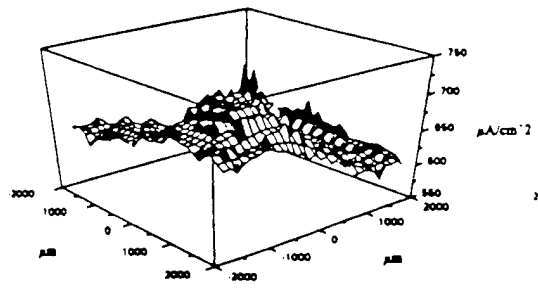

Figure 3a. (Time = 1 hr)

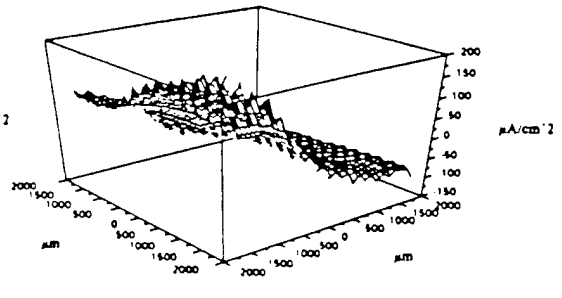

Figure 3b. (Time = 3 hr)

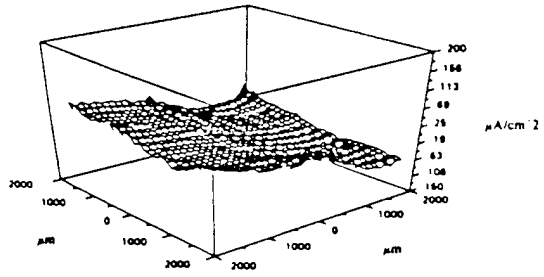

Figure 3c. (Time = 20 hr)

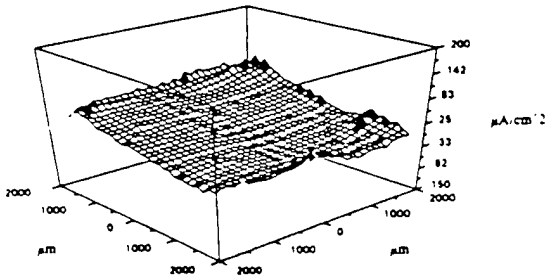

Figure 3d. (Time = 24 hr)

Figure 3. Current density (z axis) scans resulting from the addition of a concentrated drop (1 µl) of a 24 hour culture of Pseudomonas aeruginosa FRD1 placed on a mild steel coupon.

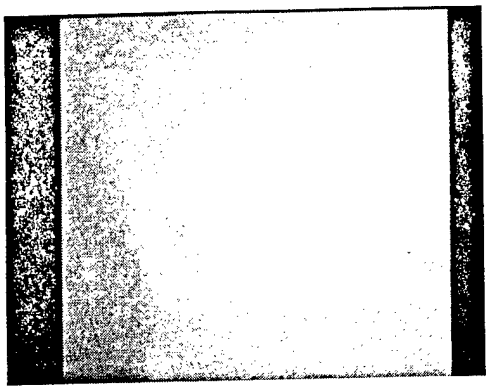

Figure 4a.

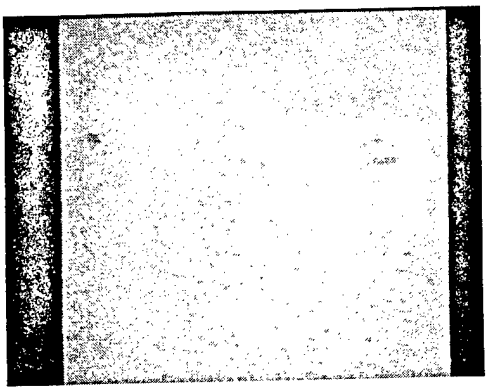

Figure 4b

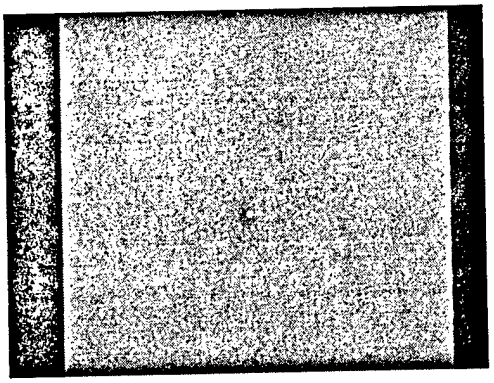

Figure 4c.

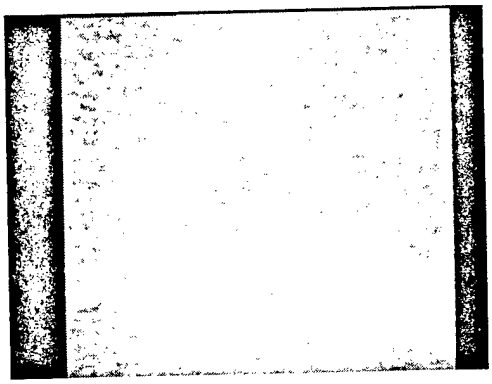

Figure 4d

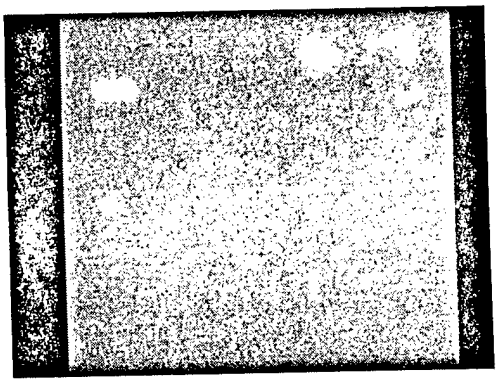

Figure 4e.

Figure 4 Images of Pseudomonas aeruginosa FRD1 on mild steel coupon: a) visual image after 1 hour exposure, b) visual image after 3 hours showing spread of bacteria, c) photon image taken after 1 hr showing little light, d) visual image taken after 24 hours, e) photon image taken after 24 hours showing the bacteria are still active.

the anodic region is seen to have grown and covered a larger area reaching the edge of the area examined (3b). Again this correlates well with the visual image (4b) that shows that the bacteria and polymer have also spread to cover a similar area. The direction of spread is parallel to the direction of flow and it is thought that the flow of the medium through the system has facilitated the spread of the bacteria. For the corresponding time periods figure 4c shows that after one hour there are a few bright areas where the bacteria are active. After three hours more light areas were visible which again showed good correlation with the visual image of where the majority of the bacteria were.

By twenty-hours this anodic region had disappeared (3c) the visual image (4d) shows that by this time the bacteria and the polymer have spread over the whole surface along with corrosion products. At twenty-four hours the results were principally the same with little anodic activity over the surface (3d) but the photon image (4e) shows that there was a lot of bacterial activity and that the alginate pathway was indeed active. This state persisted until the end of the experiment after forty-two hours.

Once again it was evident that initially there was some anodic activity which could be attributed to the location of the bacteria but was short lived as the bacteria colonised more of the surface of the coupon. The photon images indicate that the bacteria were still active after the anodic activity had halted. The link between the light and the production of alginate was demonstrated by this lab (unpublished data).

One of the major questions regarding the use of the scanning vibrating electrode technique is whether it can detect the current through a biofilm. It is arguable with these results that the anodic current was still present but could not be detected through the biofilm and corrosion products. In order to determine if this was the case a set of experiments were run using tradition dc polarisation techniques to determine the corrosion rates of mild steel electrochemically in the presence of the bacteria FRD1 and in a sterile control.

Figure 5. shows the inverse of the polarisation resistance plotted against time for both the sterile control and the FRD1 inoculated cells. Both of conditions follow similar trends with an initial increase in the rate of corrosion which then drops before a slight increase at the end of the eight day run. The initial rise in the sterile control would appear to be larger than the FRD1 cell, but proportionally to the starting value they are similar. These results correlate well to the findings in the SVEM, where initially corrosion (anodic current) was see for the first couple of days which then decreased in magnitude.

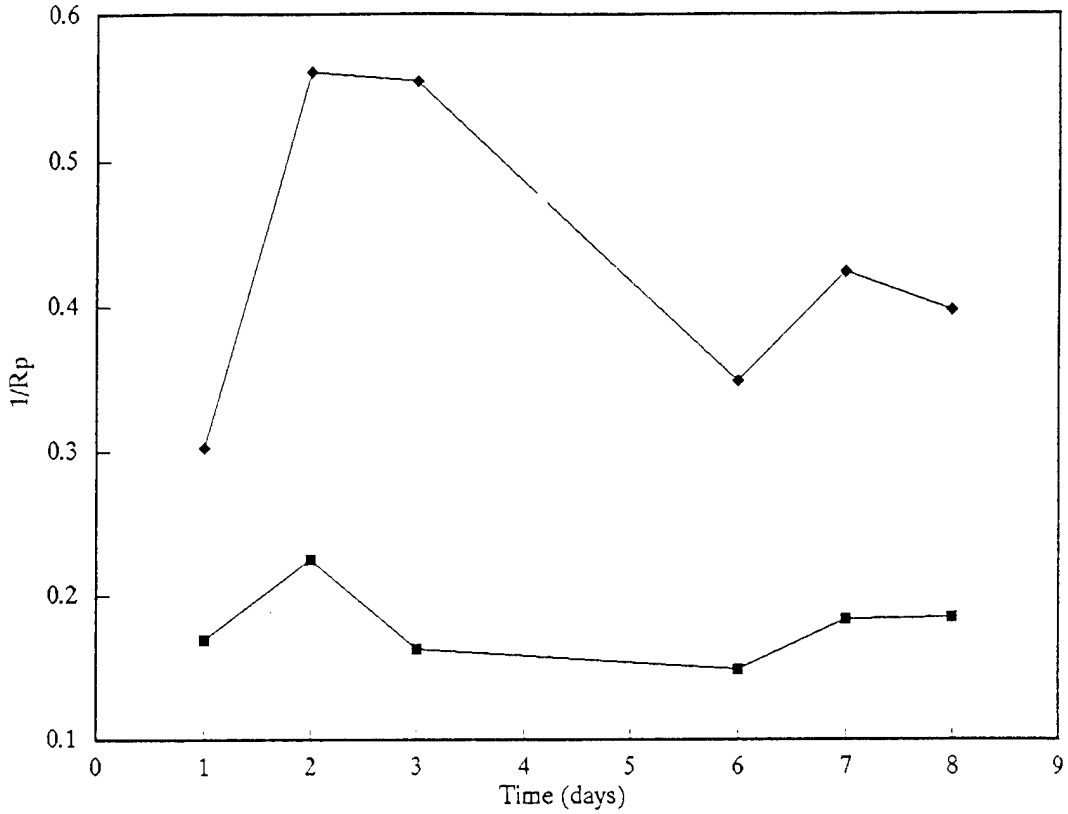

Figure 5. Time plot of the inverse of the polarisation resistance of mild steel coupons exposed to *Pseudomonas aeruginosa* FRD1 (■) and sterile control (◆).

Conclusion

It is suggested that the bacteria Pseudomonas aeruginosa FRD1 and in pa 'cular alginate are not of themselves capable of microbial induced corrosion. It is thought that the initial corrosion is due to the bacteria forming a heterogenous biofilm that allows differential oxygen cells to be set up. These electrochemical cells are not capable of fully establishing themselves into self sustaining pits and are rapidly passivated as the bacteria further grows to cover the surface.

The SVEM has also been demonstrated to be a very useful tool providing further insight into the mechanisms of microbially induced corrosion providing information on both the location and magnitude of anodic regions on a metal surface. The SVEM is able to provide this information at a resolution not previously possible with traditional electrochemical methods.

Acknowledgments

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